Bronchiolitis: Analysis of 10 Consecutive Epidemic Seasons

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Summary. Bronchiolitis is the leading cause of hospitalization in infants under 12 months. Our aims were to analyze epidemiological characteristics of infants with bronchiolitis over 10 consecutive seasons and to evaluate whether there are any clinical differences between infants hospitalized for bronchiolitis during epidemic peak months and infants in non-peak months. We enrolled consecutive enrolled 723 previously healthy term infants hospitalized at the Paediatric Emergency Department, "Sapienza" University of Rome over the period 2004–2014. Fourteen respiratory viruses were detected from nasopharyngeal aspirates by molecular methods. Clinical and demographic data were extracted from clinical charts. Viruses were detected in 351 infants (48.5%): RSV in 234 (32.4%), RV in 44 (6.1%), hBoV in 11 (1.5%), hMPV in 12 (1.6%), co-infections in 39 (5.4%), and other viruses in 11 (1.5%). Analyzing the 10 epidemic seasons, we found higher incidence for bronchiolitis every 4 years with a peak during the months December–January. Infants hospitalized during peak months had lower family history for asthma ($P = 0.003$), more smoking mothers during pregnancy ($P = 0.036$), were slightly higher breastfed (0.056), had lower number of blood eosinophils ($P = 0.015$) and had a higher clinical severity score ($P = 0.017$). RSV was detected mostly during peak months, while RV was equally distributed during the seasons. We found some variations in bronchiolitis incidence during epidemics, and discriminative characteristics in infants hospitalized for bronchiolitis during peak months and in non-peak months, that might reflect two different populations of children. **Pediatr Pulmonol. 2016; 9999:XX–XX**.

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INTRODUCTION

Acute bronchiolitis is the most common lower respiratory tract infection in infants and the leading cause of hospitalization in this group of patients.[1]

Bronchiolitis is diagnosed mostly in winter, in particular between October and May during respiratory viruses circulation periods. It is well known that the number of infants with bronchiolitis rapidly increases from the beginning of the epidemic, it reaches a peak with a plateau during the 2–3 central months and then rapidly decreases during the last months. The identification of a virus is higher during the peak months of the epidemic and it is lower at the beginning and at the end, but it is not clear whether patients who have bronchiolitis during the peak months have different demographic and clinical characteristics, comparing to those who have bronchiolitis during the non-peak months.[1,2] Furthermore, it has been shown that also the number of patients with bronchiolitis may change from 1 year to the other probably due to viruses' characteristics and the immunological status of

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Conflicts of interest: None.

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the patients.[3] Because of the conflict that exists in the definition, a more homogenous material, following strict inclusion criteria are needed.

The evident relationship between respiratory syncytial virus (RSV) and bronchiolitis has been widely described in the last decades.[4] Recently, with the improvement in viral identification techniques, it has been showed that also other respiratory viruses are associated with this disease, but the clinical characteristics of infants with bronchiolitis from other respiratory viruses is still controversial.[5–8]

The main purpose of our study was to describe the prevalence and the epidemiology of 14 respiratory viruses in previously healthy full term infants hospitalized for bronchiolitis during 10 consecutive annual epidemic periods. In addition we tested the hypothesis that infants hospitalized during the peak months of bronchiolitis epidemics were different from those hospitalized during the non-peak months, by comparing demographic and clinical characteristics in those two groups of infants.

MATERIALS AND METHODS

Patients

We prospectively enrolled 723 consecutive infants (median age 2.13 months, range [0.23–11.97], 395 [54.6%] male) hospitalized for bronchiolitis in the Paediatric Emergency Department of "Sapienza" University of Rome during 10 consecutive annual epidemic periods from October to May, from the years 2004 to 2014.

Acute bronchiolitis was clinically defined as the first episode of acute lower respiratory tract infection in infants less than 12 months, characterized by the acute onset of cough, tachypnoea, retraction, and diffuse crackles on chest auscultation.[9] Exclusion criteria were prematurity and underlying chronic diseases, such as cystic fibrosis, interstitial lung disease, congenital heart disease, and immunodeficiency.

Detailed demographic characteristics were obtained from parents with a structured questionnaire; clinical and laboratory data were extracted from patients' medical files. Studied variables included age, gender, breast feeding history, family smoking habit, school attendance by siblings, family history for asthma and atopy, blood eosinophil count, chest radiological findings, and number of days of hospitalization. In addition, a clinical severity score ranging from 0 to 8 was assigned to each infant on admission in the hospital according to respiratory rate, arterial oxygen saturation on room air, presence of retractions, and ability to tolerate oral feeding.[10]

Considering the high prevalence of bronchiolitis during specific months of the years, we divided our patients in infants who were hospitalized during the peak months and infants who were hospitalized during the non-peak months. For peak months, we empirically considered the 3 central months of each epidemic season (including at least 75% of the population/year).

The parents of all infants were asked to participate in the study and gave informed consents. The study was approved by the research and ethics committee of the Hospital.

Virus Detection

To detect respiratory viruses, we collected nasopharyngeal aspirates within the first day of hospitalization using 3 ml of sterile isotonic solution injected into each nostril and aspirated back with a syringe. We sent the samples on ice to the virology laboratory to analyze: 200 μl of respiratory specimens were used to extract nucleic acid with the total nucleic acid isolation kit (Roche Diagnostics, Mannheim, Germany), eluting with 50 ml of the supplied elution buffer. A panel of either reverse transcriptase (RT) or nested PCR assays has been used to detect 14 respiratory viruses: RSV, Rhinoviruses (RV), influenza virus (IV) A and B, human Coronavirus (hCoV) OC43, 229E, NL-63 and HUK1, Adenovirus, RV, Parainfluenza virus (PIV) 1–3, and Human Metapneumovirus (hMPV).[11] Human Bocavirus (hBoV) was detected with a different PCR method used by Allander et al.[12] Rhinoviruses were detected targeting the 5′ UTR region, well conserved among Rhinoviruses and respiratory Enteroviruses so that they are not distinguished by this test. A subset of RV-positive samples was amplified and sequenced in less conserved regions (VP4-VP2) and 1 out of 38 resulted to be Enterovirus 68.

Statistical Analysis

For statistical reasons, we decide to consider occasional viruses all the viruses found with incidence lower than 1% in the 10 years. Continuous variables values were expressed as mean ± SD or median and range and categorical variables as number and percentages. A one-way analysis of variance (ANOVA) and Student's *t*-test were used for the comparison of continues variables. The Mann–Whitney and Kruskal–Wallis tests were used to analyze categorical independent variables. We considered statistically significant a $P < 0.05$. Statistical analysis was performed using the SPSS Software (version 21.0; SPSS Inc., Chicago, IL).

RESULTS

Only 6.5% of parents approached declined to take part to the study. Among the 723 previously healthy, term, infants consecutively hospitalized for bronchiolitis, we were able to identified 391 viruses from the nasopharyngeal aspirates of 351 children. RSV was identified in 234 infants, RV in 44, hBoV in 11, hMPV in 12, PIV in 5 (four

PIV 3 and one PIV 1), hCoV in 2, IV in 4; coinfections were identified in 39 infants (RSV-RV n = 13, RSV-hBoV n = 13, RSV-Influenza n = 2, RSV-hMPV n = 2, RSV-PIV 3 n = 1, hBoV-hMPV n = 1, hBoV-PIV 1 n = 1, RV-hMPV n = 4, RV-PIV 3 n = 1, RSV-RV-hMPV n = 1).

Comparing clinical characteristics of bronchiolitis from different viruses, we found that RSV patients were significantly younger (vs. hBoV $P < 0.001$) and had a higher severity score with respect to other groups ($P < 0.041$). A higher number of blood eosinophils was found in RV patients than in other groups ($P < 0.014$). No differences were found in terms of length of hospitalization, demographic data, and clinical severity score between different viruses' groups, including coinfected infants.

Considering the 10 epidemics, we observed a significantly higher number of infants hospitalized with bronchiolitis in the 3rd (n = 102) and 7th (n = 105) epidemics (2006–2007 and 2010–2011) than in the 4th (n = 47) and 8th (n = 73) epidemics (2007–2008 and 2011–2012) ($P < 0.001$) (Fig. 1a).

Most of the patients were recruited in the winter months, especially in December–February and, analyzing viruses' detection, we found that RSV follows the temporal trend of the disease, while other viruses were equally distributed over the year (Fig. 1b). Comparing the epidemiology over the 10 years, a variability of the month with the higher incidence, even within the winter months, was found. Particularly, the month with a higher number of hospitalization for bronchiolitis was January in seven epidemics (3rd–5th, 7th–10th) and February in three (1st, 2nd, and 6th) (Fig. 2).

Dividing infants according to the hospitalization during the peak months or non-peak months, we found some statistically significant differences in terms of risk factors for respiratory diseases. The 596 infants hospitalized in the peak months had less frequently both parents with asthma ($P = 0.003$), had higher maternal smoke during pregnancy ($P = 0.036$) and were slightly higher breastfed ($P = 0.056$). Moreover, children in the peak months had lower number of eosinophils ($P = 0.015$) and had a higher severity score ($P = 0.017$) than infants in non-peak months.

Analyzing viruses' detection, RSV was more frequently detected in peak months than non-peak months, differing from RV ($P = 0.029$). No differences were found for other clinical variables evaluated (Table 1).

DISCUSSION

With the present study, we examined the epidemiology and the characteristics of acute bronchiolitis, in infants hospitalized in a single Italian pediatric medical center, over 10 consecutive epidemic seasons. The peak of hospitalization for bronchiolitis tended to come early to January and showed an increase of cases every 4 years. RSV was confirmed as the most common agent of bronchiolitis, followed by RV, hBoV, and hMPV; coinfections have been detected in 5.4% of children. In our study, we confirmed that RSV infection was present in younger and more clinically severe infants, while infants with RV had higher number of blood eosinophils. Finally, differences in terms of demographic and clinical characteristics have been found between infants hospitalized for bronchiolitis during the peak months and ones during non-peak months.

One strength of our study is that we included only infants with a well-characterized definition of

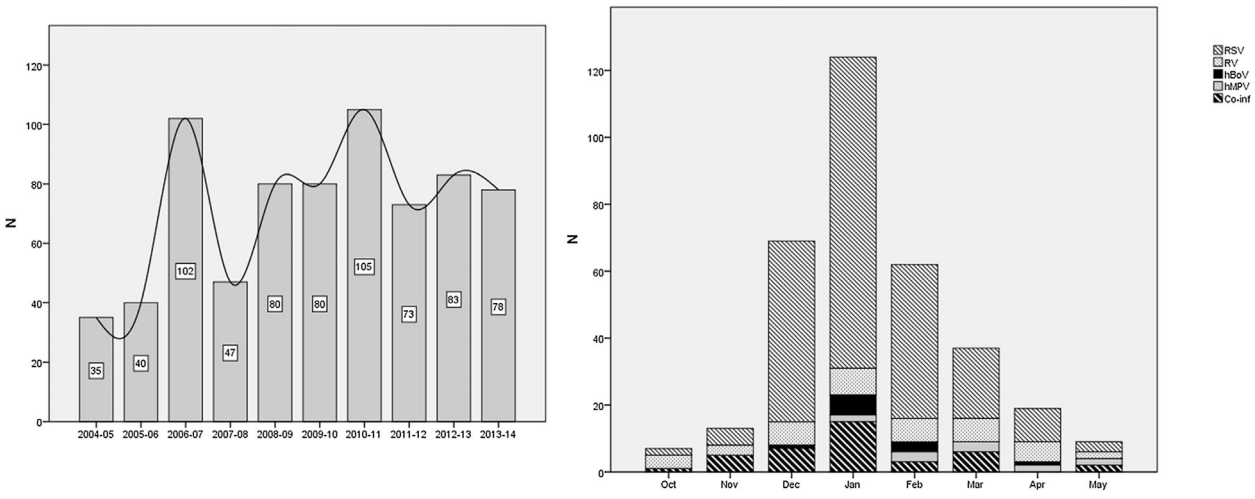

Fig. 1. (a) Number of infants hospitalized for bronchiolitis over 10 epidemics. (b) Number of infants hospitalized for bronchiolitis by infection type (RSV, RV, hBoV, hMPV, and coinfections) during the 10 epidemics divided by month.

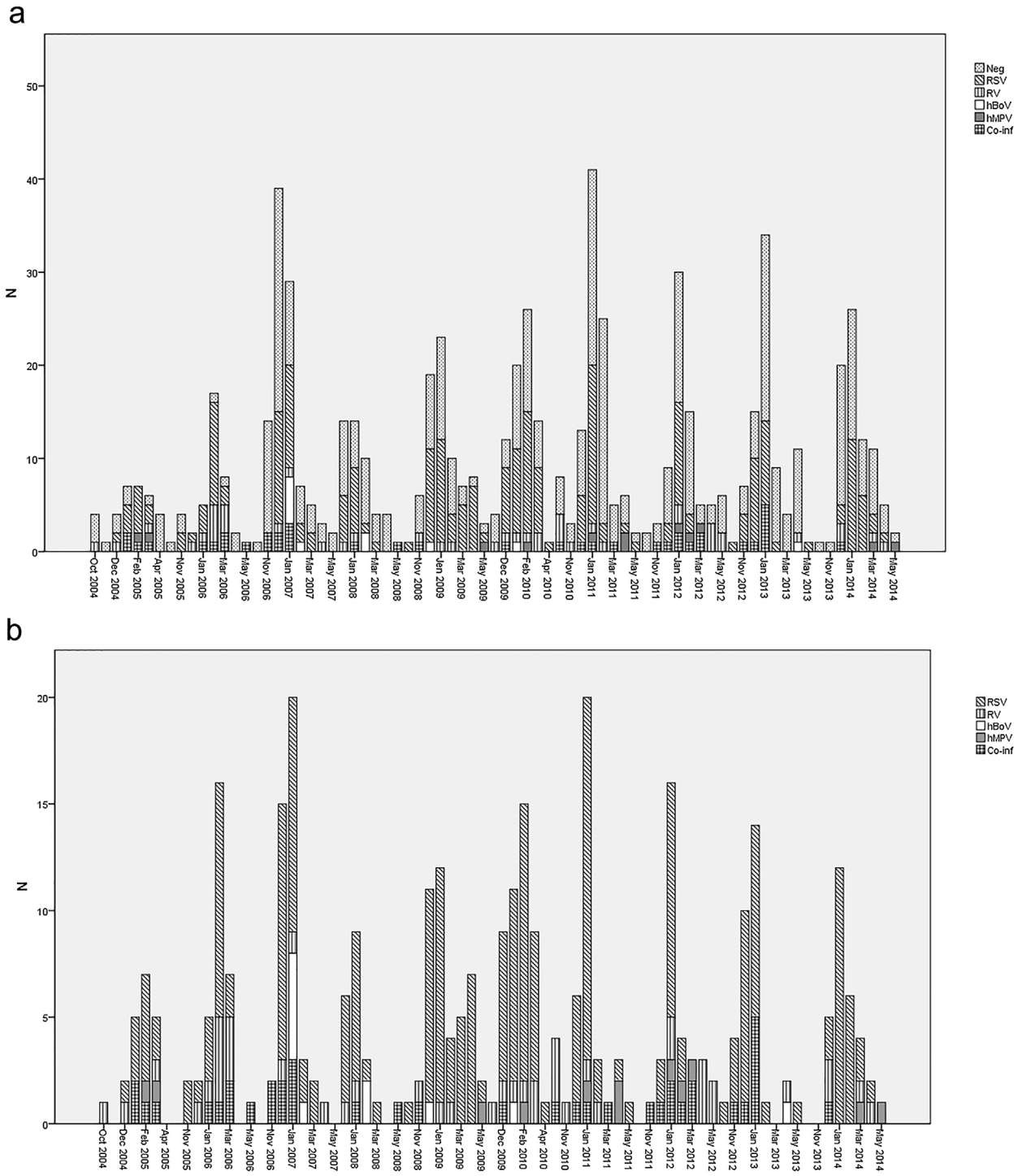

Fig. 2. Distribution of identified viruses in infants hospitalized for bronchiolitis over the 10 epidemics without (a) and with (b) negative infants.

bronchiolitis (infants younger than 12 months presenting with their first episode of lower respiratory infection, who had diffuse crackles on auscultation). Otherwise, the American Pediatric Academy defines bronchiolitis as the first episode of acute viral wheeze occurring in infants less than 2 years.[1] In order to exclude infants with virus associated episodes of wheezing, having wheezing alone was not considered sufficient for inclusion in the study.[10]

Comparing hospitalization for bronchiolitis during different years, we found a peak of incidence in the 3rd and 7th seasons in respect to the subsequent year indicating that respiratory virus circulation apparently

TABLE 1— Demographic and Clinical Variables of Infants Hospitalized for Bronchiolitis, During Peak and Non-Peak Months

Variables	Peak-months (n = 596)	Non-peak months (n = 127)	*P*
Sex (male)	54%	57.5%	ns
Age (days)	77.45 ± 58.49	83.34 ± 56.14	ns
Gestational age (weeks)	38.81 ± 1.26	38.84 ± 1.10	ns
Family history of asthma	21.3%	25.2%	ns
Both parents with positive history of asthma	0.3%	3.8%	0.003
Family history of atopy	34.4%	33.9%	ns
Presence of passive smoke	46.3%	43.7%	ns
Smoking during pregnancy	9.8%	4%	0.036
Caesarian section	51.8%	46.8%	ns
Breastfeeding at recovery	78%	70.1%	0.056
Chest x-ray consolidation	55.2%	55.8%	ns
WBC	11,589 ± 4,657	11,821 ± 5,591	ns
Blood eosinophils[1]	80 (0–1,412)	120 (0–1,315)	0.015
C-reactive protein (mg/dl)	0.44 (0–16.98)	0.3 (0–7.18)	ns
Severity score:			
Time 0	3.31 ± 2.08	2.79 ± 1.98	0.017
Time 24 hr	2.28 ± 1.83	1.55 ± 1.56	0.001
Time 48 hr	1.59 ± 1.61	0.96 ± 1.12	0.001
Days of hospitalization	5.13 ± 2.44	4.75 ± 1.79	ns
Negative for viruses	300 (50.3%)	72 (56.7%)	ns
RSV only	204 (34.2%)	30 (23.6%)	0.022
RV only	32 (5.4%)	12 (9.4%)	0.067
Bocavirus	11 (1.8%)	0	ns
Metapneumovirus	8 (1.3%)	4 (3.1%)	ns
Others	8 (1.3%)	3 (2.4%)	ns
Coinfections	33 (5.5%)	6 (4.7%)	ns

Data were expressed as mean ± SD.
Peak months were January–March for the 1st, 2nd, 6th epidemic seasons and December–February for the 3rd–5th, 7th–10th epidemic seasons.
[1]Data were expressed as median and range.

increase every 4 years. Alternating circulation of RSV types A and B could explain different rates of hospitalization in different epidemic seasons, but unfortunately, as stated below as a study limitation, we did not test for viruses' subtypes along the whole study period. Moore et al. studied duration of immunity after RSV infection assuming the need of a sufficient number of infected people at the same time to gain population immunity also considering that immunity could wane several months before the onset of a subsequent RSV season. Differences in viral circulation during the years could be linked to the circulation of different viral genotypes or, than, to the "herd immunity."[8,13] In fact, the spread of a new RSV A subtype (ON1) has been recently demonstrated in Rome.[14]

Our finding of an anticipation of the month with the higher incidence of bronchiolitis (December or January instead of March) could be linked to changes in climatic conditions, such as temperature or humidity, and to air pollution modification over different years.[15] This fact could influence viral spread along with predisposition of patients to respiratory infection.

Comparing bronchiolitis from different viruses in 10 consecutive seasons, we confirmed RSV as more severe agent affecting younger infants. Moreover, while RV was equally distributed during the year, most of RSV was detected during winter months, strongly correlating with bronchiolitis incidence trend, as other studies have already shown.[16] We think that this fact may be caused by climate factors, such as temperature, air pressure, and pollution modifications could modulate even the seasonality of viruses, as other studies already evidenced.[17,18] In fact, the study by Hervás et al. concluded that the mean temperature and atmospheric pressure were the main factors for RSV activity.[18]

Observing the distribution of bronchiolitis during the year, we were able to isolate peak and non-peak months of hospitalization for this disease with some differences between the two populations. An association between peak bronchiolitis, incidence, and clinical severity was found. It could be linked to our finding of higher incidence of RSV in peak months with respect to the non-peak months. Having both parents with asthma and the number of eosinophils were significantly lower during peak months. These facts may justify the different characteristics of this population hospitalized in a period with lower viruses detection and less severe viral infections. On the other hand, analyzing risk factors for respiratory diseases, infants exposed to maternal smoke during pregnancy were hospitalized more during peak months; this could be linked to modification of respiratory mucosa and subsequent predisposition to viral infection, in particular from RSV in at risk infants. Noakes et al. studied that smoke increases early susceptibility to infection and, then, the subsequent IgA responses.[19–23]

Our study has some limitations. First, as we discussed in our previous article,[10] although the sensitive and comprehensive PCR method, our virus detection rate was poor. It could be partly due to technical problems related to collecting and storing samples. Otherwise, some of the virus negative cases may be related to undetected pathogens. Finally, we did not test for viruses subtypes.

In conclusion, we found some variations in bronchiolitis incidence during epidemics, and discriminative characteristics in infants hospitalized for bronchiolitis during peak months and in non-peak months that might reflect two different populations of children.

AUTHORS' CONTRIBUTIONS

Cangiano G: drafting the work and approved the final manuscript as submitted. Frassanito A and Nenna R: interpretation of data for the work and approved the final

manuscript as submitted. Evangelisti M, Ambra N, Petrarca L, and Papoff P: acquisition of the data and approved the final manuscript as submitted. Pierangeli A, Scagnolari C, and Antonelli G: performed viral analysis and approved the final manuscript as submitted. Capocaccia P: reevaluated the chest X-ray. Moretti C: revising the work critically for important intellectual content and approved the final manuscript as submitted. Midulla F: conception and design of the work and approved the final manuscript as submitted.

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